Overwintering Tadpoles and Loss of Fitness Correlates In *Polypedates braueri* Tadpoles That Use Artificial Pools In A Lowland Agroecosystem

OVERWINTERING TADPOLES AND LOSS OF FITNESS CORRELATES IN *POLYPEDATES BRAUERI* TADPOLES THAT USE ARTIFICIAL POOLS IN A LOWLAND AGROECOSYSTEM

Juei-Ling Hsu[1], Yeong-Choy Kam[1,3], and Gary M. Fellers[2]

[1]*Department of Life Science, Tunghai University, Taichung 407, Taiwan*
[2]*U.S. Geological Survey, Western Ecological Research Center, Point Reyes National Seashore, Point Reyes, CA 94956, USA*

ABSTRACT: We studied growth, development, and metamorphic traits of *Polypedates braueri* tadpoles in Taiwan to elucidate the cause of tadpole overwintering in man-made water containers in lowland orchards on the Bagua Terrace. *Polypedates braueri* bred from March to August, but tadpoles were present year round. Laboratory experiments demonstrated that tadpole overwintering was facultative; low temperatures and limited food retarded both growth and development, resulting in overwintering in the tadpole stage. Tadpoles at the lowest experimental temperature (15°C) never reached metamorphosis. A field experiment demonstrated that 78, 28, and 4% of tadpoles raised in high, medium, and low food regimes, respectively, metamorphosed before the onset of winter. Tadpoles that did not metamorphose by fall continued to grow slowly and either metamorphosed during the winter or the following spring. These findings indicate that food availability plays a key role in inducing overwintering in tadpoles. Jumping performance of metamorphs was positively correlated with food regimes, but body lipid content was significantly higher in metamorphs raised with either low or high food regimes than in those with medium levels of food. Overwintering by *P. braueri* tadpoles has not been previously reported; however, agricultural activities have created new breeding habitats (i.e., man-made bodies of water), some of which are sufficiently food-limited that tadpoles overwinter to complete development and metamorphosis. An understanding of the survivorship, life history traits, and physiology of these frogs is needed to shed light on how man-made breeding sites affect the population dynamics of native frog populations.

Key words: Amphibian; Life history traits; Metamorphosis; Overwinter; Postmetamorphic performance

THE CONVERSION of natural habitats to agricultural land is one of the most significant impacts affecting amphibians throughout the world (Zug et al., 2001; Gallant et al., 2007; Wells, 2007). In Taiwan, 23% of the land is used for agriculture, and the proportion is much higher in lowland areas where the human population density is high. For example, 59% and 63% of the land is farmed in Chunghua and Yunlin counties, respectively (Council of Agriculture, 2009). Altered landscapes affect the survival, movement (Joly et al., 2001; Mazerolle, 2001; Stevens et al., 2004; Neckel-Oliveira and Gascon, 2006; Rothermel and Semlitsch, 2006), and life history traits of amphibians (Gill, 1978; Sinsch, 1992; Neckel-Oliveira, 2004; Neckel-Oliveira and Gascon, 2006). However, some agricultural activities create new habitats that benefit amphibians. For example, rice paddies provide extensive wetland habitats, and the adjacent terrestrial areas often harbor a rich biological diversity (Bambaradeniya and

Amarasinghe, 2003). Some anurans (frogs and toads) have benefited from the creation of rice fields, which they use for breeding habitat. In Taiwan, at least 38% (11 of 29) of anuran species breed in paddy fields (Shang et al., 2009), and the situation is probably similar in many parts of Asia, where many anurans readily adapt to man-made bodies of water (Berry, 1975; Bambaradeniya, 2000). On a smaller scale, the practice of growing bamboo in montane areas creates bamboo stumps that hold water and become ideal breeding sites for Eiffinger's Treefrog, *Chirixalus eiffingeri* (Kam et al., 1996).

Although amphibians are often attracted to unnatural habitats, these habitats differ in size, distribution, and physical features in ways that could influence the behavior, life history, morphology, and physiology of frogs. For example, in many lowland areas of western Taiwan, natural habitats have been converted to rice paddy fields. In those areas, the breeding pattern of the Alpine Cricket Frog (*Fejervarya limnocharis*) has been strongly influenced by agricultural practices;

[3] CORRESPONDENCE: e-mail, biyckam@thu.edu.tw

F. limnocharis breed during the rainy season, but the immediate stimulus for mating is the flooding of paddy fields by farmers (Alexander et al., 1979).

In foothills and terraces, where irrigation is difficult, most agricultural crops consist of fruit tree orchards. The Bagua Terrace in the coastal region of west-central Taiwan is a tableland (100–400-m elevation) that has been largely converted to Lychee Fruit orchards (*Litchi chinensis*; Yen et al., 2004). Concrete tanks, typically holding 500–5000 L, have been built in the orchards to collect rainwater for irrigation, and to provide a source of water for mixing with pesticides, which are then sprayed on the orchards. In many of these orchards, man-made tanks provide suitable breeding habitat for frogs. *Polypedates braueri* (Spot-Legged Treefrog) is a rhacophorid frog with expanded toe tips that enable it to climb in and out of concrete water tanks (Wu et al., 2007). We have recorded *P. braueri* foam nests and tadpoles in water tanks throughout orchards on the Bagua Terrace. Normally *P. braueri* breeds in the spring or summer, and tadpoles metamorphose before winter (Yang, 1998; Hsu et al., 2006); however, we have found *P. braueri* tadpoles in water tanks on the Bagua Terrace throughout the winter.

The purpose of our study was to describe the breeding ecology of *P. braueri*, to elucidate the cause of overwintering in tadpoles occupying man-made water containers, and to evaluate how overwintering might affect individual fitness. Specifically, we (1) documented breeding phenology, prevalence of overwintering tadpoles, and tadpole development; (2) assessed the influence of temperature and food on both growth and metamorphosis in the laboratory; (3) conducted a field experiment to evaluate the factors that might induce overwintering; and (4) measured the lipid content and jumping performance of metamorphs.

MATERIALS AND METHODS
Study Site and Species

Our study sites were at Anshilieu (24°04′N, 120°35′E; datum = WGS84) and Zuhsinken (24°04′N, 120°35′E) on the northern Bagua Terrace, an elongated tableland that runs north–south in the coastal region of Changhua County in central Taiwan. The area receives approximately 128 cm of rain annually, which mostly falls during the wet season from March through August. This low-elevation terrace (100–400-m elevation) is characterized by orchards, and early- and mid-succession woodlands (Yen et al., 2004). The terrace is one of the few remaining wooded areas in the western coastal plain of Taiwan, though the woodland is highly fragmented. The nearest weather station (Wuchi, 20 km NW of our study area) reported mean monthly air temperatures that ranged from 15.8°C in February to 29.0°C in July, with an annual mean of 22.8°C (Central Weather Bureau, http://www.cwb.gov.tw/).

Polypedates braueri is a mid-sized tree frog (snout–vent length 4–6 cm) in the family Rhacophoridae that is distributed throughout much of eastern Asia (Kuraishi et al., 2011). In Taiwan, *P. braueri* is commonly found in lowland areas where it inhabits woods, orchards, and bamboo groves. Its breeding season is March through September (Yang, 1998; Hsu et al., 2006), and foam nests are deposited where they overhang ponds, wetlands, rice fields, or man-made bodies of water. Clutch size ranges from 400 to 500 eggs (Yang, 1998); upon hatching, tadpoles drop into the water and continue to grow until metamorphosis. The main breeding habitats of *P. braueri* on the Bagua Terrace are man-made water containers such as rain-collecting reservoirs, tanks, and containers that are situated in orchards surrounded by urban areas and rice fields at the base of the terrace.

Field Surveys

We documented the breeding activities of *P. braueri* in 14 man-made containers by conducting monthly surveys from September 2003 to March 2006. Twelve of the containers were concrete and either square (length 1.0–2.4 m, height 1.0–1.5 m) or round (diameter 1.8 m, height 1.7 m); one container was plastic and one was porcelain (diameter 0.8–1.1 m, height 0.6–0.9 m). Most of the containers filled with rainwater that was funneled into the container with a loosely placed aluminum sheet, which also served to reduce evaporation in dry periods. During each visit, we recorded

the presence of calling frogs, amplectant pairs, egg clutches, and tadpoles. We used the occurrence of advertisement calls or egg clutches as evidence of reproduction (Hsu et al., 2006).

We monitored tadpole development biweekly from 4 October 2003 to 15 September 2004 by using a dip net to collect 50 tadpoles from each container where they were present. We stopped dip netting if we did not catch any tadpoles after five sweeps (Viparina and Just, 1975). Each tadpole was put in a scaled Petri dish and photographed to document tadpole length. Tadpole developmental stage (Gosner, 1960) was recorded after examination with a ×10 hand lens. We categorized tadpoles into six categories: Gosner stages 25–26, 27–31, 32–35, 36–38, 39–41, and 42–46.

Because of malfunction and vandalism of our Hi-Low thermometers during 2003–2004, we lacked sufficient temperature data for that year. Subsequently, we used thermocouples and recorded the temperature of each container once per month during the overwintering period from 17 October 2004 to 29 March 2005. We did not measure the temperatures at other times because overwintering was the primary the focus of this study. To measure water temperature, we lowered the thermocouple into the center of each pool, 10 cm below the water surface, between 0900 and 1100 h.

Laboratory Experiments

We evaluated the effects of temperature (15, 17, 20, and 26°C) and food level (high, medium, and low) on the metamorphic rate of tadpoles using a 4×3 factorial design with 20 replicates for each treatment combination. The temperatures approximated the range of water temperature measured during our field surveys. We determined how much tadpoles ate by feeding 35 tadpoles (0.04–1.092 g) with commercial tadpole food pellets (Fwusow Brand; protein 40.2%, fiber 27.1%, fat 7.3%, ash 12.1%, calcium3.2%, and phosphorus 1.9%) ad libitum. Tadpoles were weighed before and after each feeding trial to determine how much food a tadpole would eat to become full. In subsequent experiments, we fed tadpoles high, medium, and low levels of food that equated to full, 1/2 full, and 1/4 full by body mass (Beck, 1997).

We collected three egg clutches from the field and incubated them in the laboratory with dechlorinated tap water. At Gosner stage 26, we randomly assigned tadpoles to one of 12 temperature × food treatments ($n = 20$ for each of the 12 treatments). Each replicate consisted of a tadpole that was individually kept in a plastic container (length × width × height = $12.5 \times 12.5 \times 7.5$ cm) with 500 mL of water, and covered with a transparent punctured lid to reduce evaporation. All tadpoles were reared under a 12:12 h light:dark cycle, and water was changed every 3 d. Tadpoles were fed daily with food pellets; the amount of food was based on tadpole mass and food treatment level. We monitored tadpole survival weekly until metamorphosis (Gosner stage 42, when forelimbs emerged).

Field Experiment

In September 2006, we conducted a field experiment to assess the role of food in overwintering tadpoles. We collected four egg masses from the field and incubated them in dechlorinated tap water in the laboratory. After the eggs hatched and the tadpoles reached Gosner stage 26, we randomly assigned tadpoles to one of the food treatments (i.e., high, medium, or low as described above). Each treatment had eight replicates, and each replicate consisted of a plastic 12-L container that housed 10 tadpoles. In the field, containers were placed in the shade to avoid direct sunlight, and to ensure conditions similar to the containers in the orchards. Containers were covered with nets to prevent falling leaves and predators from entering. We fed tadpoles daily with food pellets as described for the laboratory experiments above. The amount of food placed in each container was based on tadpole mass and food level treatment. We changed water weekly, at which time the containers were thoroughly scrubbed to minimize algal growth. From November 2006 to March 2007, we used temperature loggers (Hobo Stowaway, www.onsetcomp.com) in one randomly selected container from each of the three treatments to record water temperature every minute.

The experiment began 23 September 2006 and ended 180 d later on 22 March 2007. We monitored tadpole survival and recorded body

mass and development stage at least once every 10 d until metamorphosis; containers were checked more frequently as tadpoles approached metamorphosis. At Gosner stage 42, we removed tadpoles from their container, and kept each tadpole in an individual 200-mL plastic box with a moist substrate until stage 46 (Gosner, 1960); at that time we measured body length and mass.

The jumping ability of recent metamorphs was assessed after holding metamorphs at 24–25°C, and then acclimating them to 26°C for 1 h. After acclimation, we dampened the frog's limbs, placed them on a clean, flat, dry surface, and then chased them to induce an escape response. We measured maximum jump distance (length of the longest leap) and average distance jumped (mean length of 10 leaps). Metamorphs were allowed to rest 5 min between each jump. Small hops (i.e., less than five body lengths) that were clearly far below an individual's capacity were excluded (Álvarez and Nicieza, 2002). After the experiment, metamorphs were euthanized by freezing, and stored at −15°C for subsequent lipid analysis.

Lipid Analysis

We used cold extraction to recover nonpolar lipids from metamorphs (Heulett et al., 1995; Álvarez and Nicieza, 2002). Briefly, we thawed metamorphs at room temperature, and then oven-dried each individual at 37°C for 4 d until they reached a constant mass (nearest 0.01 mg). Lipids were extracted by placing metamorphs in individual 2.5-mL glass vials, adding 2 mL of petroleum ether to each vial, covering the vials for 60 min, removing the ether, and repeating for a total of six ether washes. After washing, samples were dried and reweighed. The difference in pre- and postextraction mass is the amount of lipid stored in an individual. Petroleum ether extraction removes primarily nonpolar lipids (storage), but few polar (structural) lipids (Dobush et al., 1985; Heulett et al., 1995).

Statistical Analyses

The effects of food and temperature on metamorphic rate and the length of the larval period in the laboratory experiments were evaluated with two-way ANOVA. The food × temperature interaction was significant, so we conducted a simple main-effects analysis. We used Scheffe's method for post hoc comparison because of overall significance.

For the field experiment, we analyzed the effects of food regimes on the growth and development of tadpoles with a one-way multivariate ANOVA (MANOVA). We used a chi-square test to assess the occurrence of metamorphosed tadpoles before and after winter, a one-way ANOVA to evaluate metamorphic traits of tadpoles, and an analysis of covariance to analyze jumping performance and body lipid content with body mass as a covariate (Álvarez and Nicieza, 2002).

We defined 21 March to 20 June as spring, 21 September to 20 December as fall, and 21 December to 20 March as winter. We analyzed the data using SAS software (SAS Institute Inc., 2001). All values are means ± SD; we used $\alpha = 0.05$ to evaluate statistical significance.

Results

Field Surveys

Breeding phenology.—Monthly surveys of 14 man-made water containers from September 2003 to March 2006 documented *P. braueri* calling between March and August, and foam nests from April to August of each year. By contrast, *P. braueri* tadpoles were found every month throughout the study. We searched the 14 containers for overwintering tadpoles from October to March for three consecutive years, beginning in the fall of 2003 (Table 1). During that time, 12 containers had tadpoles during October, but only containers 4 and 7 had tadpoles every year. Of the 18 occasions where we found tadpoles in October (Table 1), five (2003), two (2004), and three (2005) containers contained tadpoles of varied developmental stages during the winter.

Tadpole developmental pattern.—We were able to track the development of tadpoles in 11 containers from October to March during four winters. Because not every container had tadpoles each year, we recorded 18 container histories (Table 1). In seven of these, the number of tadpoles steadily decreased as tadpoles metamorphosed, and no tadpoles

TABLE 1.—Monthly occurrence of *Polypedates braueri* tadpoles in 11 of the 14 containers monitored from October to March in each of 4 yr, 2003–2006. No tadpoles were found from October to March in containers 12–14 during the study period. The presence of tadpoles is indicated by an X. Symbols O, N, D. J, F, and M represent October, November, December, January, February, and March, respectively.

Container number	2003			2004											2005							2006		
	O	N	D	J	F	M	//	O	N	D	J	F	M	//	O	N	D	J	F	M		J	F	M
1	X	X	X	X	X			X	X															
2	X	X	X	X																				
3								X							X	X	X	X						
4	X	X	X	X	X	X		X							X	X	X	X	X	X				
5	X	X	X	X	X																			
6	X	X	X	X																				
7	X	X	X	X	X	X		X	X	X	X	X			X									
8								X																
9								X																
10								X	X						X	X	X	X	X					
11								X	X	X	X	X												

were present by the end of November. During eight of the histories, the number and proportion of early-stage tadpoles decreased, but many of the tadpoles overwintered and some did not metamorphose until the end of March (Fig. 1a). In these containers, there were no tadpoles after March, when a new cohort of tadpoles appeared during the following breeding season in May (Fig. 1a). In three of the histories, tadpoles remained abundant and the proportion of early-stage tadpoles was relatively unchanged throughout the winter (Fig. 1b); the overwintering tadpoles mixed with a new cohort of tadpoles the following breeding season.

Water temperature.—Average water temperatures of the 11 containers from 17 October 2004 to 29 March 2005 were higher in fall than during the winter (Fig. 2). The sharp drop of water temperature on 30 December 2004 was associated with a cold front. During this part of our study, containers 7 and 11 both had overwintering tadpoles. Water temperatures in these containers were either higher (container 7) or lower (container 11) than the average (Fig. 2).

Laboratory Experiments

Tadpoles reared at 15°C grew slowly and did not metamorphose after 6 mo, at which time we terminated the experiment and excluded those tadpoles from further analyses. Tadpoles fed at high and medium food levels, and raised at 17, 20, or 26°C had high rates of metamorphosis (>75%, Table 2). Tadpoles

that were fed low food levels also had high rates of metamorphosis (94%) when raised at 20°C, but not when raised at either 17 or 26°C (Table 2).

The interaction between temperature and food level was significant ($F_{4,118} = 12.38$, $P < 0.0001$, Table 2). Within the same food regime, length of the larval period decreased as temperature increased (high food $F_{2,118} = 115.67$, medium food $F_{2,118} = 86.52$, low food $F_{2,118} = 13.53$, all $P < 0.0001$). Also, the larval period decreased as food increased for tadpoles at both 20 and 26°C food regimes ($F_{2,118} = 58.82$, $F_{2,118} = 47.91$, respectively, all $P < 0.0001$), but the effect was not significant at 17°C ($F_{2,118} = 2.16$, $P = 0.12$, Table 2).

Field Experiment

Effects of food regimes.—Food level also had a significant effect on tadpole growth and development rates in the field experiment; tadpoles fed high food levels grew and developed at the fastest rates (food level × time; MANOVA, $F_{6,38} = 14.06$, $P < 0.0001$), whereas tadpoles in the low food regime grew and developed at the slowest rates ($F_{6,38} = 24.16$, $P < 0.0001$). Tadpoles in the high food regime started to metamorphose at the end of October and peaked during November (Fig. 3). Tadpoles fed medium amounts of food started to metamorphose in November and peaked in December. Some tadpoles in the low food regime metamorphosed in November, but most of them did not reach metamorphosis until March.
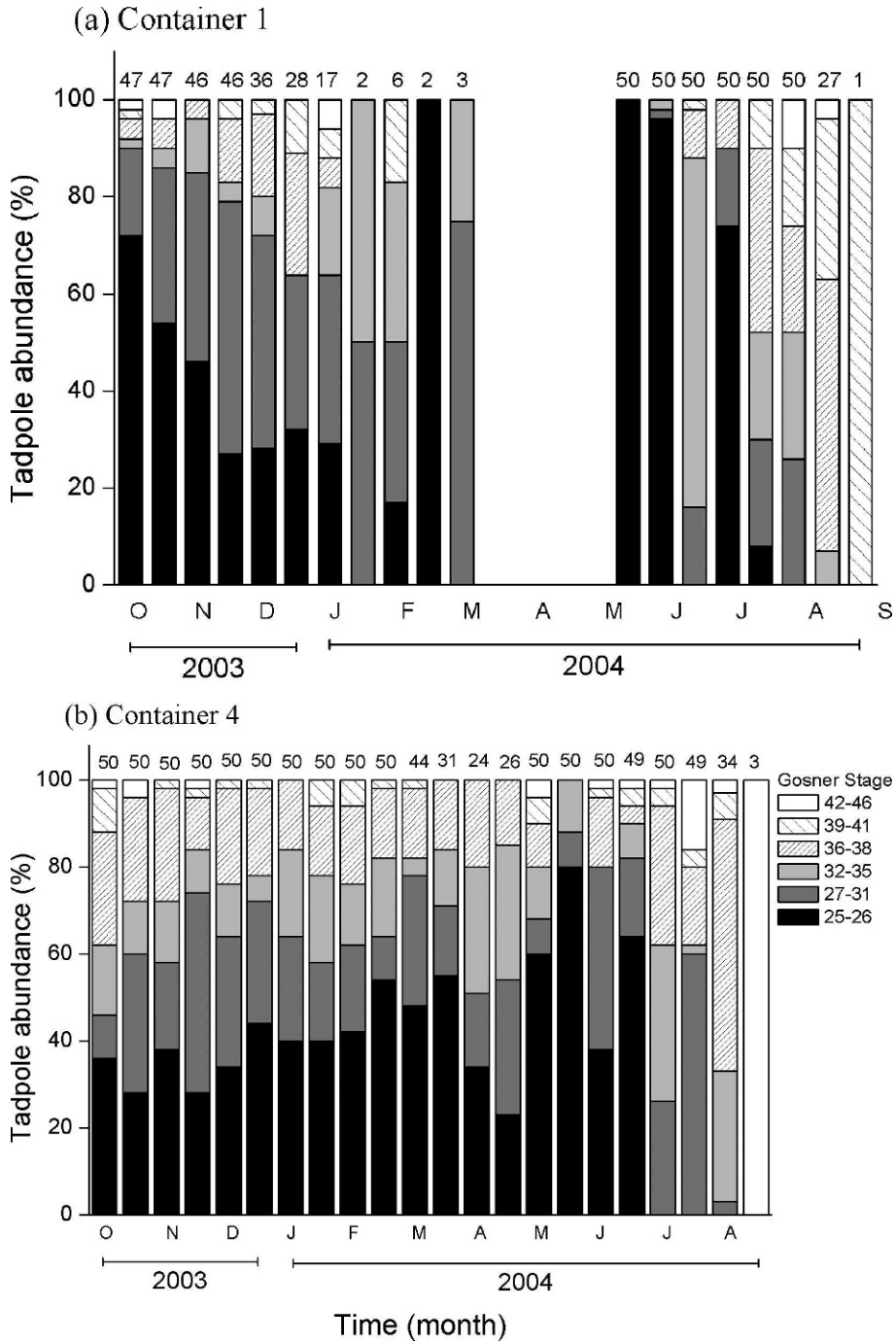
FIG. 1.—Percentage of *Polypedates braueri* tadpoles at each developmental stage in containers 1(a) and 4(b) from 4 October 2003 to 15 September 2004. The number of tadpoles in each biweekly sample is shown above each column.
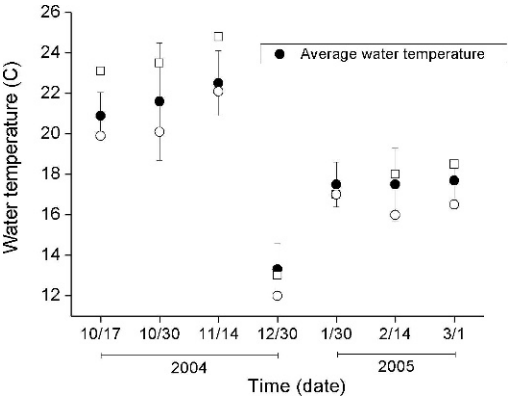
Fig. 2.—Water temperature (±SD) of 11 containers from 17 October 2004 to 29 March 2005. Containers 7 (open squares) and 11 (open circles) contained overwintering tadpoles.

Of the 80 tadpoles raised with each of the three food regimes, 66 (high), 51 (medium), and 23 (low) individuals metamorphosed by the end of experiment on 22 March 2007. A total of 62, 32, and 3 individuals from high, medium, and low food regimes, respectively, reached metamorphosis before winter began (December 21). The number of tadpoles that metamorphosed before or after December 21 was strongly dependent upon food regime (χ^2 = 54.17, df = 2, $P < 0.0001$). At the end of field study, 14 (high), 32 (medium), and 23 (low) tadpoles had died, and some tadpoles never metamorphosed (medium food, seven tadpoles, Gosner stage 33–38; low food, 34 tadpoles, Gosner stage 31–35).

Water-temperature fluctuation.—Water temperature was monitored in one randomly selected high, medium, and low food-level container throughout the field experiment. Average monthly water temperatures from 1 November 2006 to 31 March 2007 were 20.9, 17.1, 14.9, 18.1, and 17.9°C, respectively. Daily water temperature showed a sharp drop in mid-December, and stayed low until the end of February (Fig. 3). The large temperature fluctuations were associated with weather fronts. Hourly water temperatures were similar among all three containers, as shown by the low coefficients of variation (0.706% ± 1.039%, $n = 3608$).

Metamorphosis, jumping performance, and lipid content.—Tadpoles reared with high food levels reached metamorphosis the earliest, and attained the largest size; those raised in low food regimes reached metamorphosis the latest and were the smallest (Table 3). Metamorphs from the high food regimes jumped the farthest, whereas those from the low food regime jumped the shortest distances. However, adjusted body lipid content of metamorphs from the high and low food regimes was similar, and both were greater than tadpoles raised with the medium food regime (Table 3).

DISCUSSION

Overwintering Tadpoles

Polypedates braueri typically breed in natural ponds during the spring and summer, with tadpoles metamorphosing before winter (Lin and Zhang, 1990; Hsu et al., 2006). We found that *P. braueri* on the Bagua Terrace in Taiwan bred from March to August, but tadpoles were present all year, indicating that some *P. braueri* tadpoles overwinter. Overwintering is facultative because tadpoles did not overwinter every year at every breeding site. Overwintering of tadpoles in a subtropical lowland is unusual and unexpected because of the warm summers and mild winters that should allow for metamorphosis. In Taiwan, overwintering tadpoles have only been reported for *Rana sauteri* (Lai et al., 2002) and *Rana adenopleura* (Hsu et al., 2006) The population of *R. sauteri*, a stream breeder

TABLE 2.—Percentage of *Polypedates braueri* tadpoles (±SD) that metamorphosed, and the length of larval period in days (in parentheses) in the laboratory experiment. $n = 20$ tadpoles per treatment.

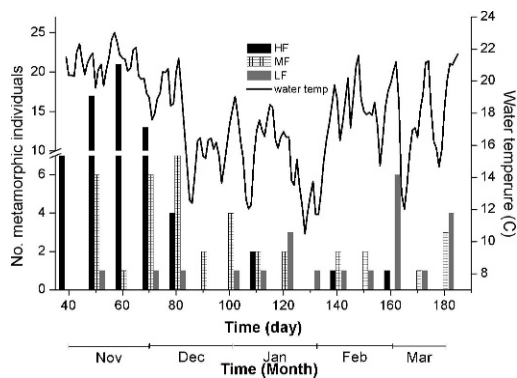| Temperatures (°C)	Food levels		
	High	Medium	Low
26	100 (31.9 ± 0.4)	100 (43.3 ± 1.8)	39 (111.4 ± 13.5)
20	84 (65.3 ± 1.7)	95 (90 ± 4.9)	94 (164.4±11.7)
17	80 (132.2 ± 4.9)	75 (131.7 ± 7.5)	40 (148.3 ± 9.5)
15	0	0	0

FIG. 3.—Number of *Polypedates braueri* tadpoles metamorphosing from high (HF), medium (MF), and low (LF) food regimes, and daily fluctuations in water temperatures over time. Daily water temperature was an average of water temperature from three recorders, one each in containers of high, medium, and low food regimes. The experiment began on 23 September 2006 and ran for 180 d.

found at high elevations (ca. 2000 m), breeds in the spring, but some tadpoles have retarded growth and development, and do not reach metamorphosis until spring of the following year (Lai et al., 2002, 2003). In contrast, lowland *R. adenopleura* breed from spring to summer, but tadpoles that hatched late in the breeding season routinely overwintered.

Overwintering tadpoles have been reported in at least 17 anuran genera and more than 40 species, including *Alsodes gargola* (Logares and Úbeda, 2004), *Ascaphus truei* (Brown, 1990), *Calyptocephallela gayi* (Diaz and Valencia, 1985), *Lithobates blairi* (Bragg and Dowell, 1954), and *Rana draytonii* (Fellers et al., 2001). *Rana sierrae* tadpoles at high-elevation ponds and lakes in the Sierra Nevada mountains of California overwinter one to three times before they metamorphose, whereas tadpoles at low elevations do not

typically overwinter (Vredenburg et al., 2005). *Lithobates catesbeianus* is a widely distributed species in central and eastern North America; tadpoles at high latitudes overwinter once or twice, but they do not overwinter at low latitudes (Collins, 1979). These same patterns have been reported for other ranid frogs as well (Cory, 1962; Berven et al., 1979; Lannoo, 2005).

Food Availability and Overwintering Tadpoles

Temperature, length of growing season, timing of breeding, and resource availability can influence whether tadpoles overwinter (Collins, 1979; Brown, 1990; Bury and Adams, 1999), but in most instances when overwintering has been reported, there is no information on what the causative factors might be. In our field experiment, the proportion of overwintering *P. braueri* tadpoles was higher when food was low, indicating that food has a significant influence on time to metamorphosis. Tadpoles reared in the high food regime grew and developed rapidly, and most of them reached metamorphosis before winter. Tadpoles with medium levels of food grew more slowly and developed more slowly, and only some of the tadpoles metamorphosed before winter. Those tadpoles that overwintered probably did so because they did not reach the minimum size for metamorphosis before the onset of winter (Wilbur and Collins, 1973; Collins, 1979). Overwintering *P. braueri* tadpoles continued to grow and develop, but at a slow rate due to the low winter temperatures. Whereas some individuals reached metamorphosis during the winter, others never did. The extreme case was tadpoles raised in the low food regime. Only a few individuals reached metamorphosis before winter, and even fewer metamorphosed during the

TABLE 3.—Size, jumping performance, and lipid content of *Polypedates braueri* metamorphs from the field experiment. Each treatment consisted of eight containers with 10 tadpoles each. Different letters represent significant differences between treatments.

Variables	High food (*n* = 8)	Medium food (*n* = 8)	Low food (*n* = 8)	*F*	*P*
Snout–vent length (cm)	1.89 ± 0.02a	1.71 ± 0.02b	1.65 ± 0.02b	26.13	< 0.0001
Body mass (mg)	535 ± 16a	437 ± 27b	421 ± 25b	8.07	0.0029
Larval period (days)	64.8 ± 3.1a	99.9 ± 5.4b	140.7 ±10.2c	29.86	< 0.0001
Adjusted jumping distance (cm)	26.24 ± 0.55a	19.21 ± 0.48b	15.68 + 0.59c	70.98	< 0.0001
Adjusted maximum jumping distance (cm)	28.57 + 0.67a	20.71 + 0.58b	17.50 + 0.72c	53.42	< 0.0001
Adjusted body lipid (mg)	10.55 ± 0.83a	5.86 ± 0.94b	10.26 ± 1.83a	9.72	0.0015

winter months. Most of the tadpoles grown with low food levels grew slowly throughout the winter and 71% of tadpoles never metamorphosed or died before the end of our field experiment.

Although cool winter temperatures played a role, there are three lines of evidence that suggest that food was the key factor causing *P. braueri* tadpoles to overwinter. First, the water temperature of containers where tadpoles overwintered in 2005 was not lower than containers where tadpoles did reach metamorphosis. This suggests that something other than temperature is the critical factor. Second, the developmental patterns of tadpoles that did or did not overwinter in our field experiment were similar to the developmental patterns of tadpoles on the Bagua Terrace. Third, the containers where *P. braueri* bred on the terrace were designed to collect rainwater for irrigation or agrochemical spraying; however, organic matter in the containers was limited, and came mostly from leaves and invertebrates that entered the containers. Containers with more organic matter were less sheltered and tended to have more algal growth than those with little organic matter (Y.-C. Kam, personal observation). The variation in algal production (e.g., food) is likely to be the critical factor that regulates tadpole growth and development in these systems.

Traits and Performance of Metamorphs

Metamorphic traits of tadpoles, and jumping performance and body lipid content of metamorphs varied among the three food regimes used in our field experiment, indicating that food availability during larval development has profound effects on *P. braueri* metamorphs. Tadpoles raised with high food regimes metamorphosed earlier and attained larger sizes, and metamorphs jumped farther when compared with individuals raised with more limited food regimes. A similar result was also found in *Discoglossus galgano* metamorphs (Álvarez and Nicieza, 2002). Large size at metamorphosis and good jumping ability probably increase fitness by enhancing survival to maturity (Wassersug and Sperry, 1977; Berven and Gill, 1983; Smith, 1987; Berven, 1990), shortening the time to maturity (Semlitsch et al., 1988; Berven,

1990), growing to a larger size at maturity (Semlitsch et al., 1988), and greater sperm number and mobility (John-Alder and Morin, 1990; Goater et al., 1993).

On the other hand, jumping ability and body lipid content are also critical to the growth and survival of the metamorphs themselves (Pfennig, 1992; Watkins, 2001). Jumping ability affects foraging ability (Walton, 1988) and predator avoidance of metamorphs (Wassersug and Sperry, 1977). Body lipid content affects whether metamorphs could survive during periods of little or no food (Pfennig, 1992; Emlet and Sadro, 2006). In our study, tadpoles raised in the high food regime were larger at metamorphosis, had high lipid content, and had better leaping ability. By contrast, tadpoles raised in the low or medium food regime metamorphosed at small size with different physiological traits in terms of body lipid content and jumping performance, which may be due to differential allocation of energy to development, growth, and lipid storage. However, how well these smaller metamorphs survive in the winter remains unclear.

Life History Traits in Altered Ecosystems

Our study demonstrated that human agricultural activities have created new breeding habitats, but the habitats are typically so food limited that *P. braueri* tadpoles often overwinter and form a new cohort when they metamorphose the following spring. These changes in breeding phenology have important ecological and conservation implications. Previous studies have shown that human agricultural activities affect not only amphibian survival and movement (Joly et al., 2001; Mazerolle, 2001; Stevens et al., 2004; Neckel-Oliveira and Gascon, 2006; Rothermel and Semlitsch, 2006), but also life history traits (Gill, 1978; Sinsch, 1992, 1997; Neckel-Oliveira and Gascon, 2006). Gill (1978) and Sinsch (1992, 1997) reported that human activities reduced breeding success of amphibians. In the human-disturbed forests, foam nests of *Phyllomedusa tarsius* have low hatching rates (Neckel-Oliveira, 2004) and male frogs have smaller body sizes (Neckel-Oliveira and Gascon, 2006).

In Taiwan, *P. braueri* is widely distributed in lowland forest where human agricultural

activities are increasingly intense. As natural habitat is increasingly converted to agricultural use, we expect that more *P. braueri* will use man-made aquatic habitats. We do not have data to demonstrate whether anthropomorphic habitats are better or worse than native habitats, but concrete cisterns allow for the continued existence of *P. braueri* in areas where they might otherwise be extirpated. In a region where maintaining natural habitats is challenging, the use of anthropomorphic habitats and phenotypic flexibility allow *P. braueri* to persist in a predominantly agricultural landscape.

In our study, *P. braueri* that use man-made water containers for breeding are now producing cohorts of tadpoles with different developmental and growth rates. These frogs are recruited into a population during the winter or the following spring, and hence they encounter different environmental conditions than they have been exposed to in the past. This is likely to lead to selection for new traits and tolerances. Comparative studies on the survivorship, life history traits, and physiology of *P. braueri* that metamorphosed before, during, and after the winter would provide insight into the adaptability of subtropical frogs and raise interesting questions about how new phonological patterns affect population dynamics of existing populations.

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